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10/622,367	07/17/2003	Phillip W. Barth	10030088-1	5348

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AGILENT TECHNOLOGIES, INC.
Legal Department, DL429
Intellectual Property Administration
P.O. Box 7599
Loveland, CO 80537-0599

EXAMINER	
NOGUEROLA, ALEXANDER STEPHAN	
ART UNIT	**PAPER NUMBER**
1753	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/622,367	BARTH ET AL.	
	Examiner	Art Unit	
	ALEX NOGUEROLA	1753	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-20 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-20 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on 17 July 2003 is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date 7/17/2003.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claim Rejections - 35 USC § 103

1. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

2. The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

3. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation

under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

4. Claims 1-4, 7-15, 19, and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bohn et al. (US 7,220,345 B2) ("Bohn") in view of Deamer (US 6,428,959 B1) ("Deamer") and Su et al. (US 7,005,264 B2) ("Su").

Addressing claim 1, Bohn discloses an apparatus for passing a molecule through a nanopore, comprising:

(a) a first microfluidic channel (28) having at least two electrodes for creating an electric field for creating electrophoretic movement of the biopolymer in a first direction (implied by col. 05:66 – col. 06:05 and col. 04:40-45, which discloses creating an electrical potential across the ends of the first microfluidic channel to electrophoretically move particles, such as molecules, along the channel);

(b) a nanopore (42) in the wall of the first microfluidic channel (Figures 1 and 5A-5C and col. 01:58-63 and claim 3); and

(c) a second microfluidic channel (30) communicating with the first microfluidic channel by way of the nanopore in the wall of the first microfluidic channel (Figures 1

and 5A-5C), the second microfluidic channel having an electrode for creating electrophoretic movement of the molecule in a second direction (Figures 1 and Figure C).

Bohn does not mention (a) threading a biopolymer through the nanopore, and (b) providing a second set of electrodes in the second channel.

As for threading a biopolymer through the nanopore, it should be first noted that this limitation is an intended use that does not further structurally limit the apparatus of Bohn. Additionally, as taught by Deamer and Su, for example, threading a biopolymer through a nanopore while using an electrical field to move the biopolymer may be achieved simply by making the nanopore small enough so that the biopolymer can pass through only when elongated. See in Deamer col. 04:38-63 and in Su col. 05:03-17. *Alternatively*, Deamer discloses threading a biopolymer through a nanopore using an electrical field. See the abstract and Figures 2A and 2B. Su also discloses threading a biopolymer through a nanopore using an electrical field. See the abstract and Figure 2. it would have been obvious to one with ordinary skill in that art at the time of the invention to substitute the nanopore membrane of Deamer or Su, such as shown in Figure2A or 2B of Deamer with the adjacent patch clamp electrodes, or as shown in Figure 2 of Su, with associated optical elements, for the membrane in Bohn because then nucleic acid hybridization events could be monitored or nucleic acids could be readily sequenced at the membrane, after undergoing a first separation in the first microfluidic channel. See in Deamer col. 01:24-34 and col. 02:19-34 and in Su the abstract and col. 03:20-32. It should be noted in this regard that Bohn already

contemplates complex interaction of the molecules with the nanopores through which they pass as Bohn discloses that the nanopores may be coated with reagent. See col. 05:39-42. Thus, Bohn discloses an interactive nanopore membrane. Also, it should be noted that Bohn already discloses performing detection at the membrane. See col. 08:30-36.

As for providing a second set of electrodes in the second channel it should be first noted that as indicated above Bohn discloses providing at least one electrode in the second channel. It would have been obvious to one with ordinary skill in the art at the time of the invention to provide at least a second electrode in the second channel, and so provide a set of electrodes in the second channel, as done in the first channel, because this will allow the molecules to undergo another separation and so further resolve the different types of molecules from each other. Indeed, Bohn implicitly contemplates this as "... a high resolution second-dimensional separation" is disclosed. See col. 06:62-66.

Addressing claim 2, Bohn discloses an apparatus for passing a molecule through a nanopore, comprising:

(a) a substrate (20) having a channel wall (28) and a nanopore (42) in the channel wall, the channel wall and nanopore being designed for receiving a molecule (Figures 1 and 5A-5C and col. 01:58-63 and claim 3);

(b) means for moving the molecule toward the nanopore in a first direction (implied by col. 05:66 – col. 06:05 and col. 04:40-45, which discloses creating an electrical potential across the ends of the first microfluidic channel to electrophoretically move particles, such as molecules, along the channel); and

(c) means for moving the molecule through the nanopore in a second direction (element (50). *Alternatively*, it should be first noted that as indicated above Bohn discloses providing at least one electrode in the second channel. It would have been obvious to one with ordinary skill in the art at the time of the invention to provide at least a second electrode in the second channel, and so provide a set of electrodes in the second channel, as done in the first channel, because this will allow the molecules to undergo another separation and so further resolve the different types of molecules from each other. Indeed, Bohn implicitly contemplates this as "... a high resolution second-dimensional separation" is disclosed. See col. 06:62-66.)

Bohn does not mention (a) threading a biopolymer through the nanopore, and (b) providing a second set of electrodes in the second channel.

As for threading a biopolymer through the nanopore, it should be first noted that this limitation is an intended use that does not further structurally limit the apparatus of Bohn. Additionally, as taught by Deamer and Su, for example, threading a biopolymer through a nanopore while using an electrical field to move the biopolymer may be achieved simply by making the nanopore small enough so that the biopolymer can pass through only when elongated. See in Deamer col. 04:38-63 and in Su col. 05:03-17. *Alternatively*, Deamer discloses threading a biopolymer through a nanopore using an

electrical field. See the abstract and Figures 2A and 2B. Su also discloses threading a biopolymer through a nanopore using an electrical field. See the abstract and Figure 2. it would have been obvious to one with ordinary skill in that art at the time of the invention to substitute the nanopore membrane of Deamer or Su, such as shown in Figure2A or 2B of Deamer with the adjacent patch clamp electrodes, or as shown in Figure 2 of Su, with associated optical elements, for the membrane in Bohn because then nucleic acid hybridization events could be monitored or nucleic acids could be readily sequenced at the membrane, after undergoing a first separation in the first microfluidic channel. See in Deamer col. 01:24-34 and col. 02:19-34 and in Su the abstract and col. 03:20-32. It should be noted in this regard that Bohn already contemplates complex interaction of the molecules with the nanopores through which they pass as Bohn discloses that the nanopores may be coated with reagent. See col. 05:39-42. Thus, Bohn discloses an interactive nanopore membrane. Also, it should be noted that Bohn already discloses performing detection at the membrane. See col. 08:30-36.

Addressing claim 3, Bohn discloses an apparatus for passing a molecule through a nanopore, comprising:

(a) a substrate (20) having a channel wall (28) and a nanopore (42) in the channel wall, the channel wall and nanopore being designed for receiving a molecule (Figures 1 and 5A-5C and col. 01:58-63 and claim 3);

(b) at least one set of electrodes for moving the molecule toward the nanopore in a first direction past the nanopore (implied by col. 05:66 – col. 06:05 and col. 04:40-45, which discloses creating an electrical potential across the ends of the first microfluidic channel to electrophoretically move particles, such as molecules, along the channel); and

(c) at least one set of electrodes for moving the molecule through the nanopore in a second direction after the biopolymer has moved past the nanopore (element (50). *Alternatively*, it should be first noted that as indicated above Bohn discloses providing at least one electrode in the second channel. It would have been obvious to one with ordinary skill in the art at the time of the invention to provide at least a second electrode in the second channel, and so provide a set of electrodes in the second channel, as done in the first channel, because this will allow the molecules to undergo another separation and so further resolve the different types of molecules from each other. Indeed, Bohn implicitly contemplates this as "... a high resolution second-dimensional separation" is disclosed. See col. 06:62-66.)

Bohn does not mention (a) threading a biopolymer through the nanopore after it has moved past the nanopore.

As for threading a biopolymer through the nanopore after it has moved past the nanopore, it should be first noted that this limitation is an intended use that does not further structurally limit the apparatus of Bohn. Additionally, as taught by Deamer and Su, for example, threading a biopolymer through a nanopore while using an electrical field to move the biopolymer may be achieved simply by making the nanopore small enough so that only the desired biopolymer can pass through. See in Deamer col. 04:38-63 and in Su col. 05:03-17.

Addressing claim 4, for the additional limitation of this claim see the abstract in Deamer and Su.

Addressing claims 7 and 8, for the additional limitations of these claims see in Deamer the abstract and Figures 2A and 2B, and in Su see the abstract and col. 02:49-62.

Addressing claims 9-13, for the additional limitations of these claims see in Bohn Figure 1. For claims 11 and 13 note that whet the first channel is above the second channel or vice versa is arbitrary as the apparatus of Bohn could be used upside down.

Indeed, the labels "first channel" and "second channel" are arbitrary in the context of this claim. Channel 30 can be construed as the first channel for claim 11 and as the second channel for claim 13.

Addressing claims 14 and 15, for the additional limitations of these claims note that Bohn only mentions a channel width of 100 micrometers and a depth of 30 micrometers as example dimensions. See col. 03:63-67. Barring a contrary showing the clamed channel dimensions are just a matter of scaling the channel for the expected volume of fluid to be contained by the channel, which will be proportional to the expected sample volume.

Addressing claims 19 and 20, for the additional limitations note that Bohn discloses making the top and bottom substrates of the apparatus from PDMS, which is transparent. See col. 03:54-57 and the second column in Siao ("Microfluidic: New Channels for Biological Research," Harvard Science Review, fall 2006, pp. 46-49), which is only cited to show a property of a material. Bohn also discloses optical detection on either side of the nanopores. See col. 08:30-36

5. Claims 5 and 6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bohn et al. (US 7,220,345 B2) ("Bohn") in view of Deamer (US 6,428,959 B1) ("Deamer") and Su et al. (US 7,005,264 B2) ("Su") as applied to claims 1-4, 7-15, 19, and 20 above, and further in view of Lockhart (US 6,344,316 B1) ("Lockhart").

Addressing claim 5, Bohn as modified by Deamer and Su does not mention having the biopolymer comprising a leader molecule attached to the biopolymer for threading the biopolymer through the nanopore.

Lockhart (which is referred to in Su, col. 14:28-41) discloses end-labeling a nucleic acid. See the abstract. It would have been obvious to one with ordinary skill in the art at the time of the invention to end label a biopolymer as taught by Lockart in the invention of Bohn as modified by Deamer and Su because as taught by Lockhart among other advantages the disclosed end –labeling allows monitoring the expression of families of closely-related nucleic acids, and permits detection of only a few copies of a nucleic acid in extremely complex acid mixtures. See col. 19:01-03 and col. 19:44-62. Also, Bohn already discloses optical detection at the nonporous membrane. See col. 08:30-41. More broadly, the use of end-labels as taught by Lockart in the invention of Bohn as modified by Deamer and Su is just a mater of using optimizing detection of the molecules as they pass through the nanopore. Note that although the leader molecules of Lockart are not for threading the biopolymer through a nanopore, this is only an intended use for which the end-labels are capable of.

Addressing claim 6, for the additional limitations of this claim see in Lockhart col. 24:37-58 and in Bohn col. 08:30-41.

6. Claims 16-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bohn et al. (US 7,220,345 B2) ("Bohn") in view of Deamer (US 6,428,959 B1) ("Deamer"), Su et al. (US 7,005,264 B2) ("Su"), Lockhart (US 6,344,316 B1) ("Lockhart"), Griffiths et al. (US 6,770,182 B1) ("Griffiths"), and Sebastin et al. ("Kramers problem for a polymer in a double well," Physical Review E, vol. 62, no. 1, pp. 927-939, July 2000) ("Sebastin") and Han et al. ("Entropic trapping and Escape of Long DNA Molecules at Sibmicron Size Constriction," Physical Review of Letters, vol. 83, no. 8, 23 August 199, pp. 1688-1689) ("Han").

Bohn discloses a method for passing a molecule through a nanopore, comprising using an apparatus that includes

(a) a first microfluidic channel (28) having at least two electrodes for creating an electric field for creating electrophoretic movement of the biopolymer in a first direction (implied by col. 05:66 – col. 06:05 and col. 04:40-45, which discloses creating an electrical potential across the ends of the first microfluidic channel to electrophoretically move particles, such as molecules, along the channel);

(b) a nanopore (42) in the wall of the first microfluidic channel (Figures 1 and 5A-5C and col. 01:58-63 and claim 3); and

(c) a second microfluidic channel (30) communicating with the first microfluidic channel by way of the nanopore in the wall of the first microfluidic channel (Figures 1 and 5A-5C), the second microfluidic channel having an electrode for creating electrophoretic movement of the molecule in a second direction (Figures 1 and Figure C).

Bohn does not mention (a) threading a biopolymer through the nanopore, (b) providing a second set of electrodes in the second channel, (c) the biopolymer having a leader molecule, and (d) threading a biopolymer through the nanopore after it passes the nanopore.

As for threading a biopolymer through the nanopore, Bohn implies using the apparatus with biomolecules, since the nanopores may be coated with chemical reagents, such as antibodies. See col. 04:50-54 and col. 05:39-45. Additionally, as taught by Deamer and Su, for example, threading a biopolymer through a nanopore while using an electrical field to move the biopolymer may be achieved simply by making the nanopore width large enough so that the biopolymer can pass through. See in Deamer col. 04:38-63 and in Su col. 05:03-17. *Alternatively*, Deamer discloses threading a biopolymer through a nanopore using an electrical field. See the abstract and Figures 2A and 2B. Su also discloses threading a biopolymer through a nanopore using an electrical field. See the abstract and Figure 2. it would have been obvious to one with ordinary skill in that art at the time of the invention to substitute the nanopore

membrane of Deamer or Su, such as shown in Figure2A or 2B of Deamer with the adjacent patch clamp electrodes, or as shown in Figure 2 of Su, with associated optical elements, for the membrane in Bohn because then nucleic acid hybridization events could be monitored or nucleic acids could be readily sequenced at the membrane, after undergoing a first separation in the first microfluidic channel. See in Deamer col. 01:24-34 and col. 02:19-34 and in Su the abstract and col. 03:20-32. It should be noted in this regard that Bohn already contemplates complex interaction of the molecules with the nanopores through which they pass as Bohn discloses that the nanopores may be coated with reagent. See col. 05:39-42. Thus, Bohn discloses an interactive nanopore membrane. Also, it should be noted that Bohn already discloses performing detection at the membrane. See col. 08:30-36.

As for providing a second set of electrodes in the second channel it should be first noted that as indicated above Bohn discloses providing at least one electrode in the second channel. It would have been obvious to one with ordinary skill in the art at the time of the invention to provide at least a second electrode in the second channel, and so provide a set of electrodes in the second channel, as done in the first channel, because this will allow the molecules to undergo another electrophoresis separation and so further resolve the different types of molecules from each other. Indeed, Bohn implicitly contemplates this as "... a high resolution second-dimensional separation" is disclosed. See col. 06:62-66.

As for the biopolymer having a leader molecule, Lockhart (which is referred to in Su, col. 14:28-41) discloses end-labeling a nucleic acid. See the abstract. It would

have been obvious to one with ordinary skill in the art at the time of the invention to end label a biopolymer as taught by Lockart in the invention of Yamakawa as modified by Bohn, Deamer, and Su because as taught by Lockhart among other advantages the disclosed end –labeling allows monitoring the expression of families of closely-related nucleic acids, and permits detection of only a few copies of a nucleic acid in extremely complex acid mixtures. See col. 19:01-03 and col. 19:44-62. Also, Yamakawa already discloses optical detection at the nonporous membrane. See the abstract and col. 06:47-64. More broadly, the use of end-labels as taught by Lockart in the invention of Yamakawa as modified Su is just a mater of using optimizing detection of the molecules as they pass through the nanopore.

As for moving the biopolymer past the nanopore before threading it through the nanopore, this is an event that will statistically occur. One way it may occur is that when the biopolymers are pulled along the first channel, say channel 104 in Yamakawa, some of the biopolymers will overshoot the nanopore 110; that is, there will be a distribution of biopolymers around the nanopore, some directly over it, some before it, and some after it. See for example Figures 2A-2D and col. 02:05-15 in Griffiths, which discloses that even at the intersection of two microchannel (effectively a micropore) it is difficult to keep the sample band thickness smaller than a channel width. So when a potential is applied to thread biopolymers through the nanopore some biopolymers will enter the nanopore from before it, some from above it, and some from past it. Another way the biopolymer may move past the nanopore before being threaded through it is that statistically some of the biopolymers will enter the nanopore sideways, through a

"hairpin crossing", such as shown in Figure 5 of Sebastin, rather than by an end crossing. Sebastin has likened a nanopore to a double well potential and worked out the activation energy required for a molecule much longer than the width of the nanopore to thread through the nanopore by end-crossing and by hairpin crossing. They found "... that the activation energy for hairpin crossing is two times the activation energy for end crossing. Inspite of this, for long enough chains, where geometry of the systems permit, hairpin formation can be the dominant mode of escape as seen in the experiments of Hans *et al.* [14]." See the abstract and the first paragraph of **VII. Conclusions**. on page 937. Han, which Sebastian refers to in the quoted passage, fond that for DNA molecules driven by an electric field through a nanometer constriction were entropically trapped at the constriction and escaped with a characteristic lifetime. "Counterintuitively, longer DNA were found to escape entropic traps faster than shorter ones." See the abstract.

7. Claims 1-4, 7-15, 19, and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yamakawa et al. (US 6,806,543 B2) ("Yamakawa"), in view of Bohn et al. (US 7,220,345 B2) ("Bohn"), Deamer (US 6,428,959 B1) ("Deamer"), and Su et al. (US 7,005,264 B2) ("Su").

Addressing claim 1, Yamakawa discloses an apparatus for passing a molecule through a nanopore, comprising:

(a) a first microfluidic channel (106) having at least two electrodes for creating an electric field for creating electrophoretic movement of the biopolymer in a first direction (implied by col. 07:58-65, which discloses generating flow of fluid and molecules through the channels by known electrokinetic or electrosmotic methods, which commonly involve applying an electrical field across a pair of electrodes separated by the length of the channel. See, for example, col. 05:61 – col. 06:05 in Bohn, which discloses an apparatus substantially the same as claimed including electrodes at the end of the first channel and implicitly at the end of the second channel. See item 4 of this Office action.);

(b) a nanopore (110) in the wall of the first microfluidic channel (Figures 1a, 1c, and 1f; and col. 03:29-41; and col. 05:25-33); and

(c) a second microfluidic channel (104) communicating with the first microfluidic channel by way of the nanopore in the wall of the first microfluidic channel (Figures 1a and 1f), the second microfluidic channel having an electrode for creating electrophoretic movement of the molecule in a second direction (implied by col. 07:58-65, which discloses generating flow of fluid and molecules through the channels by known electrokinetic or electrosmotic methods, which commonly involve applying an electrical field across a pair of electrodes separated by the length of the channel. See, for example, col. 05:61 – col. 06:05 in Bohn, which discloses an apparatus substantially the same as claimed including electrodes at the end of the first channel and implicitly at the end of the second channel, for first and second dimension electrophoresis separations. See item 4 of this Office action. See item 4 of this Office action).

Yamakawa does not mention threading a biopolymer through the nanopore, although Yamakawa does disclose using the apparatus for DNA analysis or other biological molecules. See col. 01:27-30 and col. 05: 25-33.

As for threading a biopolymer through the nanopore, it should be first noted that this limitation is an intended use that does not further structurally limit the apparatus of Yamakawa. Additionally, as taught by Deamer and Su threading a biopolymer through a nanopore while using an electrical field to move the biopolymer may be achieved simply by making the nanopore small enough so that the biopolymer can pass through only when elongated. See in Deamer col. 04:38-63 and in Su col. 05:03-17. It would have been obvious to set the diameter of the nanopores as taught by Deamer and Su in the invention of Yamakawa because Yamakawa teaches selecting a pore size from a few nanometers to micrometers to fine tune ". . . the filtration, metering, and separation of targeted chemical and biological molecules." See col. 05:25-33. *Alternatively*, Deamer discloses threading a biopolymer through a nanopore using an electrical field. See the abstract and Figures 2A and 2B. Su also discloses threading a biopolymer through a nanopore using an electrical field. See the abstract and Figure 2. It would have been obvious to one with ordinary skill in that art at the time of the invention to substitute the nanopore membrane of Deamer or Su, such as shown in Figure2A or 2B of Deamer, with the adjacent patch clamp electrodes, or as shown in Figure 2 of Su, with associated optical elements, for the membrane in Yamakawa because then nucleic acid hybridization evens could be monitored or nucleic acids could be readily sequenced at the membrane, after undergoing a first separation in the first microfluidic

channel. See in Deamer col. 01:24-34 and col. 02:19-34 and in Su the abstract and col. 03:20-32. It should be noted in this regard that Yamakawa already discloses manufacturing the nanoporous membrane as a senor "... in addition to its filtering/sieving/molecular separation capability." See col. 06:47-59.

Addressing claim 2, Yamakawa discloses an apparatus for passing a molecule through a nanopore, comprising:

(a) a substrate (100) having a channel wall (106) and a nanopore in the channel wall (Figures 1a, 1c, and 1f; and col. 03:29-41; and col. 05:25-33), the channel wall and nanopore being designed for receiving a biopolymer (Yamakawa discloses using the apparatus for DNA analysis or other biological molecules. See col. 01:27-30 and col. 05: 25-33).

Yamakawa does not disclose threading a biopolymer through the nanopore, and does not directly mention "means for moving the biopolymer toward the nanopore in a first direction; and ... means for threading the biopolymer through the nanopore in a second direction."

As for threading a biopolymer through the nanopore, it should be first noted that this limitation is an intended use that does not further structurally limit the apparatus of Yamakawa. Additionally, as taught by Deamer and Su threading a biopolymer through a nanopore while using an electrical field to move the biopolymer may be achieved

simply by making the nanopore small enough so that the biopolymer can pass through only when elongated. See in Deamer col. 04:38-63 and in Su col. 05:03-17. It would have been obvious to set the diameter of the nanopores as taught by Deamer and Su in the invention of Yamakawa because Yamakawa teaches selecting a pore size from a few nanometers to micrometers to fine tune ". . . the filtration, metering, and separation of targeted chemical and biological molecules." See col. 05:25-33. *Alternatively*, Deamer discloses threading a biopolymer through a nanopore using an electrical field. See the abstract and Figures 2A and 2B. Su also discloses threading a biopolymer through a nanopore using an electrical field. See the abstract and Figure 2. It would have been obvious to one with ordinary skill in that art at the time of the invention to substitute the nanopore membrane of Deamer or Su, such as shown in Figure2A or 2B of Deamer, with the adjacent patch clamp electrodes, or as shown in Figure 2 of Su, with associated optical elements, for the membrane in Yamakawa because then nucleic acid hybridization evens could be monitored or nucleic acids could be readily sequenced at the membrane, after undergoing a first separation in the first microfluidic channel. See in Deamer col. 01:24-34 and col. 02:19-34 and in Su the abstract and col. 03:20-32. It should be noted in this regard that Yamakawa already discloses manufacturing the nanoporous membrane as a senor "... in addition to its filtering/sieving/molecular separation capability." See col. 06:47-59.

As for providing "means for moving the biopolymer toward the nanopore in a first direction; and ... means for threading the biopolymer through the nanopore in a second direction", this is implied by col. 07:58-65, which discloses generating flow of fluid and

molecules through the channels by known electrokinetic or electrosmotic methods, which commonly involve applying an electrical field across a pair of electrodes separated by the length of the channel. See, for example, col. 05:61 – col. 06:05 in Bohn, which discloses an apparatus substantially the same as claimed including electrodes at the end of the first channel and implicitly at the end of the second channel, for first and second dimension electrophoresis separations. See item 4 of this Office action.

Addressing claim 3, Yamakawa discloses an apparatus for passing a molecule through a nanopore, comprising:

(a) a substrate (100) having a channel wall (106) and a nanopore in the channel wall (Figures 1a, 1c, and 1f; and col. 03:29-41; and col. 05:25-33), the channel wall and nanopore being designed for receiving a biopolymer (Yamakawa discloses using the apparatus for DNA analysis or other biological molecules. See col. 01:27-30 and col. 05: 25-33).

Yamakawa does not disclose threading a biopolymer through the nanopore after it has passed the nanopore, and does not directly mention "at least one set of electrodes for moving the biopolymer toward the nanopore in a first direction past the nanopore; and . . . at least one set of electrodes for moving the biopolymer in a second

direction through the nanopore after the biopolymer has been moved past the nanopore."

As for threading a biopolymer through the nanopore after it has past the nanopore, it should be first noted that this limitation is an intended use that does not further structurally limit the apparatus of Yamakawa. Additionally, as taught by Deamer and Su threading a biopolymer through a nanopore while using an electrical field to move the biopolymer may be achieved simply by making the nanopore small enough so that the biopolymer can pass through only when elongated. See in Deamer col. 04:38-63 and in Su col. 05:03-17. It would have been obvious to set the diameter of the nanopores as taught by Deamer and Su in the invention of Yamakawa because Yamakawa teaches selecting a pore size from a few nanometers to micrometers to fine tune ". . . the filtration, metering, and separation of targeted chemical and biological molecules." See col. 05:25-33.

As for providing mention "at least one set of electrodes for moving the biopolymer toward the nanopore in a first direction past the nanopore; and . . . at least one set of electrodes for moving the biopolymer in a second direction through the nanopore after the biopolymer has been moved past the nanopore", this is implied by col. 07:58-65, which discloses generating flow of fluid and molecules through the channels by known electrokinetic or electrosmotic methods, which commonly involve applying an electrical field across a pair of electrodes separated by the length of the channel. See, for example, col. 05:61 – col. 06:05 in Bohn, which discloses an apparatus substantially the same as claimed including electrodes at the end of the first channel and implicitly at the

end of the second channel, for first and second dimension electrophoresis separations. See item 4 of this Office action.

Addressing claim 4, for the additional limitation of this claim see in Yamakawa col. 01:15-30, especially lines 28-30, and col. 05:30-33. Also see the Deamer and Su abstracts. Note that for the rejection of underlying claim 3 Deamer and Su were only relied upon for disclosing how the diameter of the nanopore controls what biomolecules, such as nucleic acids, may pass through the nanopore. These references were not relied upon for the particulars of their nanopores.

Addressing claims 7 and 8, for the additional limitations of these claims see in Deamer the abstract and Figures 2A and 2B, and in Su see the abstract and col. 02:49-62.

Addressing claims 9-13, for the additional limitations of these claims see in Yamakawa Figures 1a – 1f. For claims 11 and 13 note that whet the first channel is above the second channel or vice versa is arbitrary as the apparatus of Bohn could be

used upside down. Indeed, the labels "first channel" and "second channel" are arbitrary in the context of this claim. Channel 104 can be construed as the first channel for claim 11 and as the second channel for claim 13.

Addressing claims 14 and 15, for the additional limitations of these claims note that since the apparatus is a microfluidic apparatus barring a contrary showing the clamed channel dimensions are just a matter of scaling the channel for the expected volume of fluid to be contained by the channel, which will be proportional to the expected sample volume.

Addressing claims 19 and 20, for the additional limitations note that Yamakawa discloses making the top and bottom substrates of the apparatus from PDMS, which is transparent. See col. 06:36-46 and the second column in Siao ("Microfluidic: New Channels for Biological Research," Harvard Science Review, fall 2006, pp. 46-49), which is only cited to show a property of a material. Bohn also discloses optical detection. See col. 06:60-64.

8. Claims 5 and 6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yamakawa et al. (US 6,806,543 B2) ("Yamakawa"), in view of Bohn et al. (US 7,220,345 B2) ("Bohn"), Deamer (US 6,428,959 B1) ("Deamer"), and Su et al. (US 7,005,264 B2) ("Su") as applied to claims 1-4, 7-15, 19, and 20 above, and further in view of Lockhart (US 6,344,316 B1) ("Lockhart").

Yamakawa as modified by Deamer and Su does not mention having the biopolymer comprising a leader molecule attached to the biopolymer for threading the biopolymer through the nanopore.

Lockhart (which is referred to in Su, col. 14:28-41) discloses end-labeling a nucleic acid. See the abstract. It would have been obvious to one with ordinary skill in the art at the time of the invention to end label a biopolymer as taught by Lockart in the invention of Yamakawa as modified by Deamer and Su because as taught by Lockhart among other advantages the disclosed end –labeling allows monitoring the expression of families of closely-related nucleic acids, and permits detection of only a few copies of a nucleic acid in extremely complex acid mixtures. See col. 19:01-03 and col. 19:44-62. Also, Yamakawa already discloses optical detection at the nonporous membrane. See the abstract and col. 06:47-64. More broadly, the use of end-labels as taught by Lockart in the invention of Yamakawa as modified by Deamer and Su is just a mater of using optimizing detection of the molecules as they pass through the nanopore. Note that although the leader molecules of Lockart are not for threading the biopolymer

through a nanopore, this is only an intended use for which the end-labels are capable of.

Addressing claim 6, for the additional limitations of this claim see in Lockhart col. 24:37-58 and in Yamakawa col. 06:47 - col. 07:24.

9. Claims 16 and 18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yamakawa et al. (US 6,806,543 B2) ("Yamakawa"), in view of Bohn et al. (US 7,220,345 B2) ("Bohn"), Deamer (US 6,428,959 B1) ("Deamer"), Su et al. (US 7,005,264 B2) ("Su"), Lockhart (US 6,344,316 B1) ("Lockhart"), Griffiths et al. (US 6,770,182 B1) ("Griffiths"), and Sebastin et al. ("Kramers problem for a polymer in a double well," Physical Review E, vol. 62, no. 1, pp. 927-939, July 2000) ("Sebastin") and Han et al. ("Entropic trapping and Escape of Long DNA Molecules at Sibmicron Size Constriction," Physical Review of Letters, vol. 83, no. 8, 23 August 199, pp. 1688-1689) ("Han").

Addressing claim 16, Yamakawa discloses a method of moving a biopolymer through a nanopore, comprising

(a) moving the biopolymer in a first direction; and

(b) moving the biopolymer through the nanopore in a second direction.

For the nanopore see Figures 1a, 1c, and 1f; and col. 03:29-41; and col. 05:25-33. For moving the biopolymer in a first direction and then in a second direction, particularly by electrophoresis as required by claim 18, see col. 07:58-65, which discloses generating flow of fluid and molecules through the channels by known electrokinetic or electrosmotic methods, which commonly involve applying an electrical field across a pair of electrodes separated by the length of the channel. See, for example, col. 05:61 – col. 06:05 in Bohn, which discloses an apparatus substantially the same as claimed including electrodes at the end of the first channel and implicitly at the end of the second channel. See item 4 of this Office action. For a biopolymer see col. 01:27-30 and col. 05: 25-33 in Yamakawa.

Yamakawa does not mention (a) moving the biomolecule past the nanopore in the first direction, and (b) threading the biopolymer through the nanopre in a second direction.

As for threading the biopolymer through the nanopore, as taught by Deamer and Su threading a biopolymer through a nanopore while using an electrical field to move the biopolymer may be achieved simply by making the nanopore width large enough for the biopolymer to pass through. See in Deamer col. 04:38-63 and in Su col. 05:03-17. It would have been obvious to set the diameter of the nanopores as taught by Deamer

and Su in the invention of Yamakawa because Yamakawa teaches selecting a pore size from a few nanometers to micrometers to fine tune " . . . the filtration, metering, and separation of targeted chemical and biological molecules." See col. 05:25-33. *Alternatively*, see the abstract and Figures 2A and 2B. Su also discloses threading a biopolymer through a nanopore using an electrical field. See the abstract and Figure 2. It would have been obvious to one with ordinary skill in that art at the time of the invention to substitute the nanopore membrane of Su as shown in Figure 2 of Su, with associated optical elements, for the membrane in Yamakawa because then nucleic acid hybridization events could be monitored or nucleic acids could be readily sequenced at the membrane, after undergoing a first separation in the first microfluidic channel. See in Deamer col. 01:24-34 and col. 02:19-34 and in Su the abstract and col. 03:20-32. It should be noted in this regard that Yamakawa already discloses manufacturing the nanoporous membrane as a senor "... in addition to its filtering/sieving/molecular separation capability." See col. 06:47-59.

As for the biopolymer having a leader molecule, Lockhart (which is referred to in Su, col. 14:28-41) discloses end-labeling a nucleic acid. See the abstract. It would have been obvious to one with ordinary skill in the art at the time of the invention to end label a biopolymer as taught by Lockart in the invention of Yamakawa as modified by Bohn, Deamer, and Su because as taught by Lockhart among other advantages the disclosed end –labeling allows monitoring the expression of families of closely-related nucleic acids, and permits detection of only a few copies of a nucleic acid in extremely complex acid mixtures. See col. 19:01-03 and col. 19:44-62. Also, Yamakawa already

discloses optical detection at the nonporous membrane. See the abstract and col. 06:47-64. More broadly, the use of end-labels as taught by Lockart in the invention of Yamakawa as modified Su is just a mater of using optimizing detection of the molecules as they pass through the nanopore.

As for moving the biopolymer past the nanopore before threading it through the nanopore, this is an event that will statistically occur. One way it may occur is that when the biopolymers are pulled along the first channel, say channel 104 in Yamakawa, some of the biopolymers will overshoot the nanopore 110; that is, there will be a distribution of biopolymers around the nanopore, some directly over it, some before it, and some after it. See for example Figures 2A-2D and col. 02:05-15 in Griffiths, which discloses that even at the intersection of two microchannel (effectively a micropore) it is difficult to keep the sample band thickness smaller than a channel width. So when a potential is applied to thread biopolymers through the nanopore some biopolymers will enter the nanopore from before it, some from above it, and some from past it. Another way the biopolymer may move past the nanopore before being threaded through it is that statistically some of the biopolymers will enter the nanopore sideways, through a "hairpin crossing", such as shown in Figure 5 of Sebastn, rather than by an end crossing. Sebastin has likened a nanopore to a double well potential and worked out the activation energy required for a molecule much longer than the width of the nanopore to thread through the nanopore by end-crossing and by hairpin crossing. They found "... that the activation energy for hairpin crossing is two times the activation energy for end crossing. Inspite of this, for long enough chains, where geometry of the

systems permit, hairpin formation can be the dominant mode of escape as seen in the experiments of Hans *et al.* [14]." See the abstract and the first paragraph of **VII. Conclusions**. on page 937. Han, which Sebastian refers to in the quoted passage, fond that for DNA molecules driven by an electric field through a nanometer constriction were entropically trapped at the constriction and escaped with a characteristic lifetime. "Counterintuitively, longer DNA were found to escape entropic traps faster than shorter ones." See the abstract.

Addressing claim 17, Yamakawa discloses an method for passing a molecule through a nanopore, comprising using an apparatus that includes

(a) a first microfluidic channel (106) having at least two electrodes for creating an electric field for creating electrophoretic movement of the biopolymer in a first direction (implied by col. 07:58-65, which discloses generating flow of fluid and molecules through the channels by known electrokinetic or electrosmotic methods, which commonly involve applying an electrical field across a pair of electrodes separated by the length of the channel. See, for example, col. 05:61 – col. 06:05 in Bohn, which discloses an apparatus substantially the same as claimed including electrodes at the

end of the first channel and implicitly at the end of the second channel. See item 4 of this Office action. For a biopolymer see col. 01:27-30 and col. 05: 25-33.);

(b) a nanopore (110) in the wall of the first microfluidic channel (Figures 1a, 1c, and 1f; and col. 03:29-41; and col. 05:25-33); and

(c) a second microfluidic channel (104) communicating with the first microfluidic channel by way of the nanopore in the wall of the first microfluidic channel (Figures 1a and 1f), the second microfluidic channel having an electrode for creating electrophoretic movement of the molecule in a second direction (implied by col. 07:58-65, which discloses generating flow of fluid and molecules through the channels by known electrokinetic or electrosmotic methods, which commonly involve applying an electrical field across a pair of electrodes separated by the length of the channel. See, for example, col. 05:61 – col. 06:05 in Bohn, which discloses an apparatus substantially the same as claimed including electrodes at the end of the first channel and implicitly at the end of the second channel, for first and second dimension electrophoresis separations. See item 4 of this Office action. See item 4 of this Office action).

Yamakawa does not mention (a) moving the biomolecule past the nanopore in the first direction, and (b) threading the biopolymer through the nanopore in a second direction.

As for threading the biopolymer through the nanopore, as taught by Deamer and Su threading a biopolymer through a nanopore while using an electrical field to move the biopolymer may be achieved simply by making the nanopore width large enough for the biopolymer to pass through. See in Deamer col. 04:38-63 and in Su col. 05:03-17.

It would have been obvious to set the diameter of the nanopores as taught by Deamer and Su in the invention of Yamakawa because Yamakawa teaches selecting a pore size from a few nanometers to micrometers to fine tune " . . . the filtration, metering, and separation of targeted chemical and biological molecules." See col. 05:25-33. *Alternatively*, see the abstract and Figures 2A and 2B. Su also discloses threading a biopolymer through a nanopore using an electrical field. See the abstract and Figure 2. It would have been obvious to one with ordinary skill in that art at the time of the invention to substitute the nanopore membrane of Su as shown in Figure 2 of Su, with associated optical elements, for the membrane in Yamakawa because then nucleic acid hybridization events could be monitored or nucleic acids could be readily sequenced at the membrane, after undergoing a first separation in the first microfluidic channel. See in Deamer col. 01:24-34 and col. 02:19-34 and in Su the abstract and col. 03:20-32. It should be noted in this regard that Yamakawa already discloses manufacturing the nanoporous membrane as a senor "... in addition to its filtering/sieving/molecular separation capability." See col. 06:47-59.

As for the biopolymer having a leader molecule, Lockhart (which is referred to in Su, col. 14:28-41) discloses end-labeling a nucleic acid. See the abstract. It would have been obvious to one with ordinary skill in the art at the time of the invention to end label a biopolymer as taught by Lockart in the invention of Yamakawa as modified by Bohn, Deamer, and Su because as taught by Lockhart among other advantages the disclosed end –labeling allows monitoring the expression of families of closely-related nucleic acids, and permits detection of only a few copies of a nucleic acid in extremely

complex acid mixtures. See col. 19:01-03 and col. 19:44-62. Also, Yamakawa already discloses optical detection at the nonporous membrane. See the abstract and col. 06:47-64. More broadly, the use of end-labels as taught by Lockart in the invention of Yamakawa as modified Su is just a mater of using optimizing detection of the molecules as they pass through the nanopore.

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energy for end crossing. Inspite of this, for long enough chains, where geometry of the systems permit, hairpin formation can be the dominant mode of escape as seen in the experiments of Hans *et al.* [14]." See the abstract and the first paragraph of **VII. Conclusions**. on page 937. Han, which Sebastian refers to in the quoted passage, fond that for DNA molecules driven by an electric field through a nanometer constriction were entropically trapped at the constriction and escaped with a characteristic lifetime. "Counterintuitively, longer DNA were found to escape entropic traps faster than shorter ones." See the abstract.

Claim Objections

10. Claim 1 is objected to because of the following informality: in line 7 "the the" should be -- the --. Appropriate correction is required.

11. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ALEX NOGUEROLA whose telephone number is (571) 272-1343. The examiner can normally be reached on M-F 8:30 - 5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, NAM NGUYEN can be reached on (571) 272-1342. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Alex Noguerola
Primary Examiner
AU 1753
May 23, 2007